

CLAIMS

We claim:

5 1. A purified preparation of primate embryonic stem cells which (i) is capable of proliferation in vitro culture for over one year, (ii) maintains a normal karyotype through prolonged culture, (iii) maintains the potential to differentiate to derivatives of endoderm, mesoderm, and ectoderm tissues throughout the culture, and (iv) will not differentiate when cultured on a fibroblast feeder layer.

10 2. The preparation of claim 1 wherein the stem cells will spontaneously differentiate to trophoblast and produce chorionic gonadotropin when cultured to high density.

15 3. A purified preparation of primate embryonic stem cells wherein the cells are negative for the SSEA-1 marker, positive for the SSEA-3 marker, positive for the SSEA-4 marker, express alkaline phosphatase activity, are pluripotent, and have normal karyotypes.

20 4. The preparation of claim 3 wherein the cells are positive for the TRA-1-60, and TRA-1-81 markers.

5 5. The preparation of claim 3 wherein the cells continue to proliferate in an undifferentiated state after continuous culture for at least one year.

25 6. The preparation of claim 3 wherein the cells will differentiate to trophoblast when cultured beyond confluence and will produce chorionic gonadotropin.

7. The preparation of claim 3 wherein the cells remain euploid for more than one year of continuous culture.

5 8. The preparation of claim 3 wherein the cells differentiate into cells derived from mesoderm, endoderm and ectoderm germ layers when the cells are injected into a SCID mouse.

10 9. A method of isolating a primate embryonic stem cell line, comprising the steps of:
(a) isolating a primate blastocyst;
(b) isolating cells from the inner cell mass of the blastocyst of (a);
(c) plating the inner cell mass cells on embryonic fibroblasts, wherein inner cell mass-derived cell masses are formed;
(d) dissociating the mass into dissociated cells;
(e) replating the dissociated cells on embryonic feeder cells;
(f) selecting colonies with compact morphologies and cells with high nucleus to cytoplasm ratios and prominent nucleoli; and
(g) culturing the cells of the selected colonies.

15 20 25 10. A method as claimed in claim 9 further comprising maintaining the isolated cells on a fibroblast feeder layer to prevent differentiation.

11. A cell line developed by the method of step 9.

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